



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

(of C)
R #17

In re Application of: Ralf ZINK et al.

Confirmation No.: 2908

Patent No.: 6,521,443 B1

Patent Date: February 18, 2003

Application No.: 09/559,355

Filing Date: April 27, 2000

For: GROWTH MEDIUM FOR LACTOBACILLI Attorney Docket No.: 88265-320
CONTAINING AMINO ACIDS,
NUCLEOSIDES AND IRON

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. § 1.322

Commissioner for Patents
Washington, D.C. 20231

Certificate

APR 03 2003

Sir:

of Correction

Applicants hereby respectfully request the issuance of a Certificate of Correction in connection with the above-identified patent. The corrections are listed on the attached Form PTO-1050, submitted in duplicate. The corrections requested are as follows:

At column 8, line 31 (claim 11, line 4), change "whether the milk derived base" to --wherein the milk-derived base--. Support for this correction can be found in the Examiner's Amendment at page 3 of the of the Notice of Allowability wherein the Examiner amended claim 11, line 3, to delete the phrase "whether the mild-derived base" and insert therefor --wherein the milk-derived base--.

At column 8, lines 33-34 (claim 12, lines 1-2), delete "for each nucleoside". Since this phrase was not part of applicants' original claim (see application claim 18) and it was not added by Examiner's amendment, it is believed that this phrase was inadvertently inserted during the printing of the patent.

At column 8, line 36 (claim 12, line 4), delete "each," and insert --for each nucleoside,--. At page 3 of the Notice of Allowability, the Examiner amended application claim 18, line 2, after "each" to insert the phrase "for each nucleoside", which would amend the phrase to read "each for each nucleoside,". Although applicants' agreed to this Examiner's amendment, it is believed that by deleting the first occurrence of "each" the phrase would be more grammatically correct. As this change does not affect the meaning or involve the

introduction of new matter, it is respectfully requested that the amendment be entered in this manner.

At column 8, line 38 (claim 12, line 6), change "from" to --iron--. Support for this change can be found in the Examiner's Amendment at page 3 of the Notice of Allowability.

At column 8, line 55 (claim 15, line 4), change "acid-and" to --acid and--. Support for this correction can be found in application claim 21, line 3 in applicants' Amendment and Reply filed on March 8, 2002.

No fee is believed to be due for this request. Should any fees be required, however, please charge such fees to Winston & Strawn Deposit Account No. 501-814. Please issue a Certificate of Correction in due course.

Respectfully submitted,

3/28/03

Date



Allan A. Fanucci, Reg. No. 30,256

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212-294-3311

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,521,443 B1
DATED : February 18, 2003
INVENTOR(S) : Zink et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 8,

Line 31, change "whether the milk derived base" to -- wherein the milk-derived base --;

Lines 33-34, delete "for each nucleoside";

Line 36, delete "each," and insert -- for each nucleoside, --;

Line 38, delete "from" and insert -- iron --; and

Line 55, change "acid-and" to -- acid and --.

Signed and Sealed this

Twenty-fourth Day of June, 2003

A handwritten signature in black ink, appearing to read "James E. Rogan", with a horizontal line drawn underneath it.

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

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Line 36, delete "each," and insert --for each nucleoside,--;
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Line 55, change "acid-and" to --acid and--.

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strain of cells. However, none of these fermentations reached the low pH of about 4 reached after fermenting the base supplemented with yeast extract.

TABLE 2

10% skim milk and Additives*	Aerobiosis	Anaerobiosis
Freebases	5.9	5.8
Ribonucleosides	6.3	5.6
Deoxyribonucleosides	5.8	5.9

Examples were performed with UHT whole milk gave similar results.

Results of incubation tests with a preferred combination of supplemental additives are shown in Table 3. This Table 3 contains the final pH after 24 hours incubation at 37° C. of *L. johnsonii* La I (NCC 533) and other *L. johnsonii* strains in 10% skim milk, under aerobic and anaerobic incubation conditions, and whole fat UHT milk, each supplemented with four ribonucleosides, four amino acids and ferrous sulphate. Similar results were obtained for other strains.

This data, compared to the growth data of milk without supplements and with yeast extract supplements that were shown in Table 1, shows the improved growth patterns across a wide variety of *L. johnsonii* strains. Un-supplemented 10% skim milk inoculated with *L. johnsonii* La 1 (NCC 533) reached a pH of 6.0 after 24 hours of aerobic incubation. The yeast extract supplemented 10% skim milk inoculated with *L. johnsonii* La 1 (NCC 533) reached a pH of 3.9 after 24 hours of aerobic incubation. The ribonucleosides, four amino acids and ferrous sulphate supplemented 10% skim milk inoculated with *L. johnsonii* La 1 (NCC 533) reached a pH of 4.0 after 24 hours of aerobic incubation.

TABLE 3

	NCC 533	ATCC 33200	DSM 20553	ATCC 332	ATCC 11506	DSM 33199
Aerobiosis	final pH	final pH	final pH	final pH	final pH	final pH
10% skim milk	4.0	5.4	4.2	5.1	5.3	4.5
whole fat UHT milk	3.9	5.5	4.8	6.2	5.4	4.5
Anaerobiosis						
10% skim milk	4.2	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. means not determined.

We claim:

1. A medium for growing Lactobacilli comprising: a milk-derived base; and an additive system that comprises at least four free amino acids, at least two nucleosides, and iron, in amounts sufficient in combination to promote growth of lactobacilli in the medium.
2. The medium according to claim 1, wherein the nucleosides are ribonucleosides, each added in the range of from about 10 to about 500 milligrams per liter of the medium.
3. The medium according to claim 1, wherein the nucleosides are selected from the group consisting of adenosine, guanosine, cytidine, and uridine, and wherein the at least four free amino acids added comprise cysteine.

4. The medium according to claim 3, wherein the nucleosides consist essentially of a combination of adenosine and guanosine or cytidine and uridine; or a mixture of the combination.

5. The medium according to claim 1, wherein the amount of iron added is in the range of about 10 to 200 milligrams of iron per liter of the medium.

6. The medium according to claim 1, wherein the at least four free amino acids added comprise cysteine, alanine, serine and isoleucine, each in an amount ranging from about 10 to about 200 milligrams per liter of the medium.

7. The medium according to claim 1, further comprising a compound that provides antioxidant or reducing activity.

8. The medium according to claim 7, wherein the compound that provides antioxidant or reducing activity is selected from the group consisting of cysteine, thioglycollic acid, ascorbic acid and mixtures thereof.

9. The medium according to claim 1, further comprising added magnesium and aspartic acid.

10. The medium according to claim 9, wherein the at least four free amino acids added comprise cysteine, alanine, serine and isoleucine, each in an amount ranging from about 10 to about 200 milligrams per liter of the medium; wherein the nucleosides added are each in the range of from about 10 to about 500 milligrams per liter of the medium; and wherein the iron added is in the range of about 10 to about 200 milligrams per liter of the medium.

11. The medium according to claim 1, wherein the milk-derived base comprises whole milk, partially de-fatted milk, skim milk or ultra-high temperature pasteurized milk, ~~whether the milk derived base is prepared from a natural source or from dried milk powder by addition of water.~~

12. The medium according to claim 1, wherein ~~for each nucleoside~~ said nucleosides comprise a combination of adenosine and guanosine, or cytidine and uridine in an amount of 0.1 g/l ~~each~~, and wherein said at least four free amino acids comprise alanine, serine, isoleucine and cysteine in an amount of 0.05 g/l each; and wherein said ~~from~~ is FeSO₄ in an amount of 0.1 g/l.

13. A medium for growing Lactobacilli comprising: a milk-derived base; and an additive system that comprises at least four free amino acids, at least two nucleosides selected from the group consisting of adenosine, guanosine, cytidine, and uridine each added in the range of from about 10 to about 500 milligrams per liter of the medium, and iron in the range of about 10 to 200 milligrams of iron per liter of the medium to promote growth of lactobacilli in the medium.

14. The medium according to claim 13, wherein the at least four free amino acids added comprise cysteine, alanine, serine and isoleucine, each in an amount ranging from about 10 to about 200 milligrams per liter of the medium.

15. The medium according to claim 14, further comprising a compound that provides antioxidant or reducing activity selected from the group consisting of cysteine, thioglycollic acid, ascorbic acid and mixtures thereof.

16. The medium according to claim 15, further comprising added magnesium and aspartic acid.

* * * * *

wherein the milk-derived

for each nucleoside, iron

acid and